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Cytogenetic and Molecular Studies of a Familial Paracentric Inversion of Y Chromosome Present in a Patient With Ambiguous Genitalia

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Here we describe the first reported case of a patient with a familial paracentric inversion in the long arm of the Y chromosome and ambiguous genitalia. FISH analyses with Y chromosome YACs demonstrated that the inversion breakpoints of the patients and the father's Ys appear to be the same and lie within interval 5B of the Y chromosome. PCR and sequence analysis indicated that our patient carries a normal *SRY* gene. For an additional comparison of the patient's inv(Y) with the father, two other Y chromosome sequences were examined. Molecular studies of this familial inverted Y chromosome showed no differences in the *ZFY* and *TSPY* genes between the father and the patient suggesting that the short arm of our patient's inv(Y) is identical to that of the patient's father. Southern analysis using a probe of the DAX-1 gene indicated that a single copy of DSS (dosage sensitive sex reversal) locus was present in the patient. Our results suggest that the abnormal sexual development in our patient is likely attributable to (an)other mechanism(s) than mutation in the *SRY* gene and dosage alteration of the DAX-1 gene. Am. J. Med. Genet. 70:134–137, 1997.

KEY WORDS: familial; inverted Y; ambiguous genitalia

INTRODUCTION

The pericentrically inverted Y chromosome is considered a structural variant of the human Y chromosome and is generally not associated with specific phenotypic abnormalities [Schmid, 1985]. In most cases, inverted Y chromosomes are inherited. The prevalence of males with pericentrically inverted Ys in the general population is approximately 1 per 1,000 [Zeuthen and Nielsen, 1973]. However, the paracentric inversion of the long arm of the Y chromosome is very rare and has only been described once [Madan, 1995]. Here we report a patient with ambiguous genitalia and a paracentrically inverted long arm of Y that was also present in the patient's father and grandfather. To our knowledge, this is the first reported case of an individual with abnormal sex development and an inherited inverted Y chromosome.

Although the clinical significance of a paracentrically inverted Y is unknown, the concurrence of ambiguous genitalia and a familial inv(Y) is intriguing. Since this inv(Y) is familial suggesting its benign nature, the cause of abnormal sexual development in our patient is expected to be similar to that of XY females with cytogenetically normal Y chromosomes in whom molecular changes of certain Y sequences have been described. The gene *SRY* located at distal Yp is responsible for sex-determination in mammals [Sinclair et al., 1990; Koopman et al., 1991] and its mutations have been regarded as the cause of sex reversal in some XY females [Müller et al., 1992; Mittwoch, 1992]. The function of *SRY* may be affected by the changes occurring in other Y sequences, and the development of a fertile male indeed requires multiple genes on the Y chromosome [Mittwoch 1992; Bogan and Page, 1994]. Also, deletion of other Y chromosome sequences associated with XY females have been described in humans and mice [McElreavy et al., 1992; Capel et al., 1993]. Due to the phenotype of our patient, the inv(Y) is suspected to be molecularly different than that in the proband's father and grandfather. In this study, we describe our molecular analyses of *SRY* sequences and of two other Y genes, zinc finger protein Y (*ZFY*) [Page et al., 1987]

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and testis-specific protein Y-encoded (*TSPY*) [Zhang et al., 1992], of this familial inverted Y chromosome. Our results showed that there is neither change in the coding *SRY* sequence nor any gross alteration of the *ZFY* and *TSPY* genes between the father and the proband. In addition, mapping with selected Y-specific yeast artificial chromosome (YAC) clones revealed that the inversion breakpoints of their Ys appear identical and lie between YACs yOX 85 and yOX 215, within the interval 5B [Foote et al., 1992].

X-linked sex reversal genes have been implicated in several familial cases of XY sex reversal [Wachtel and Simpson, 1994]. Recently, the DAX-1 gene (dosage sensitive sex reversal-adrenal hypoplasia congenita critical region on the X chromosome, gene 1) was isolated [Muscatelli et al., 1994]. Double dosage of this dosage-sensitive sex reversal (DSS) locus found in some XY females was thought to be responsible for male to female sex reversal [Bardoni et al., 1994]. Southern analysis showed the presence of a single copy of DAX-1 gene in the proband. These findings suggest that some other mechanism(s) beside mutation in the SRY gene and duplication of DSS is responsible for the abnormal sexual development in our patient.

MATERIALS AND METHODS

Clinical Report

The 2-year-old baby was born at 39 weeks of gestation via breech delivery to a G2P1 white woman. Ambiguous genitalia, a single opening on the perineum, and non-palpable gonads were noted at birth. The genitalia consisted of a phallus of 2 cm in stretched length with chordee. There was a very small perineal opening with non-pigmented labia/scrotal folds without gonads palpable. A urogenital sinus, vagina and cervical dimple were noted by retrograde genitogram. Due to inadequate phallus size and the presence of normal Mullerian structures, the decision was made to raise the baby as a female.

Vaginoplasty with clitoral reduction was performed at age 2 months. At 18 months, the patient underwent gonadectomy. Findings at the time of surgery included a small atrophic uterus with normal appearing fallopian tubes and intraabdominal gonads which histologically consisted of testes without epididymis or vas deferens.

Cytogenetic and Molecular Studies

G-, Q- and C-banding studies were performed on phytohemagglutinin-stimulated peripheral blood lymphocytes from the patient and her father and paternal grandfather according to standard procedures [Verma and Babu, 1989]. Tissue biopsies from skin and each gonad were obtained at the time of gonadectomy and processed for chromosome analysis. Lymphocytes from the patient and her father were transformed by Epstein-Barr virus, and these lymphoblastoid cell lines were used for fluorescence in situ hybridization (FISH) and molecular studies. FISH with biotinylated probes was carried out as previously described [Shaper et al., 1992].

Several Y-specific probes were used for Southern blotting and FISH analyses. The hYfin probe is a 1.3 Kb genomic fragment harboring the last exon of the human *ZFY* gene [Nagamine et al., 1989]. It hybridizes with 3.5 Kb and 1.8 Kb EcoRI fragments corresponding to the chromosomes Yp and Xp, respectively. The Y231 cDNA of 1.1 Kb encodes the repeated *TSPY* gene localized to the proximal region of Yp [Zhang et al., 1992]. The Y231 cosmid, used for FISH experiments, contains two 20 Kb repeat units of the *TSPY* gene. YAC clones yOX 85 and yOX 215, spanning the interval 5A–5C of Y, were selected based on the physical contig map of the human Y chromosome [Foote et al., 1992] and used to delineate the breakpoints of the inv(Y)s of the patient and her father. The clone yOX 85 harbors Y centromere and its adjacent Yq sequences and is proximal to yOX 215.

The *SRY* coding sequences were amplified from respective genomic DNAs by PCR with *SRY*-5 primer, 5′-GGCCGAATTCATGCAATCATATGCTTCGC-3′, and *SRY*-3 primer, 5′-GGCCGAATTCGGTCTTTGTAGCCAATGTTA-3′ [Coward et al., 1994]. The resulting PCR products were sequenced directly using internal primers and the chain termination reaction as described previously [Su and Lau, 1993]. For the *SRY* promoter fragments, the SRY-P5′-R1 primer, 5′-GGCCGAATTCATGTTAGCCATCCTAGAAGTTGG-3′ and SRY-P3′-BamHI primer, 5′-GGCCGGATCCCTATCCAAAACTCACTTCTACCA-3′ were used in the PCR amplification of genomic DNA.

A 389 bp fragment corresponding to nucleotides 515–883 of the DAX-1 gene [Zanaria et al., 1994] was amplified by PCR and used as a probe for Southern analysis. The primer pairs used for PCR were 1AaF, 5′-CGAAGGCGCCCGAGGCGAC-3′, and 1AaR, 5′-CGC TTGATTTGTGCTCGTGG-3′. PCR amplification was carried according to the published procedure [Zanaria et al., 1994]. A 2.5 kb cDNA probe of the phospholipase Cδ1 gene on chromosome 3 [Cheng et al., 1995] was used for Southern hybridization as a control indicator for the amount of DNA loaded in each lane.

RESULTS

Cytogenetic analysis performed on Giemsa-banded chromosomes from lymphocytes revealed a paracentric inversion in the long arm of the Y chromosome [46,X,inv(Y)(q11q21)] in the patient, her father and grandfather. This inversion was verified by Q- and C-banding showing the heterochromatin (Yqh) of Yq being adjacent to the centromere instead of its ordinary distal position (Fig. 1). FISH analyses with Y chromosome YACs indicated that the inversion breakpoints in

Fig. 1. G-, Q- and C-banded paracentrically inverted Y chromosome [inv(Y)(q11q12)].

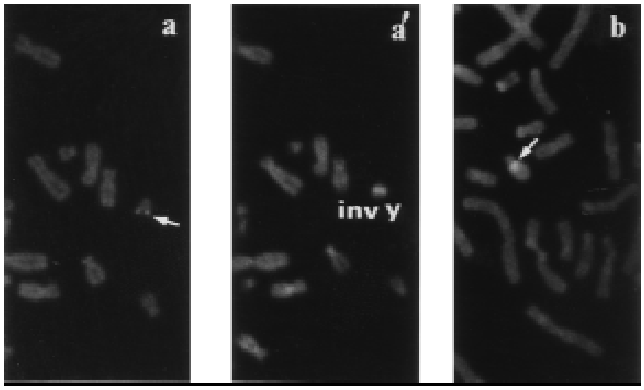

Fig. 2. Representative metaphases from our patient (**a, a'**) and a normal male (**b**) after FISH with YAC yOX 215. (a') DAPI stain for inv(Y) identification.

both the patient's and her father's Y chromosomes lie between YACs yOX 85 and yOX 215 within interval 5B. As shown in Fig. 2, the signal of yOX 215 is adjacent to the centromere of a normal Y and is at the distal Yq of the inv(Y). To rule out the possibility of mosaicism in which the other cell population(s) was not detected in lymphocytes, 50 cells from cultures of each of the patient's gonadal and skin biopsies were analyzed and the inv(Y) was present in every cell.

The presence of *SRY* in the patient was shown by PCR amplification of coding and promoter regions revealing fragments of normal size (0.63 kb for the coding region; 0.53 kb for promoter) similar to that observed in her father (Fig. 3a). Furthermore, the patient, her father and a normal control were found to contain identical DNA sequences of SRY coding and promoter regions [Su and Lau, 1993].

Deletion of other Y chromosome sequences has been implicated in XY female sex reversal. The possibility that a cytogenetically undetectable pericentric inversion may disrupt the sequences adjacent to centromere was explored. FISH with a Y-231 cosmid to metaphases prepared from the patient's and her father's lymphoblastoid cell lines showed positive hybridization signals at the proximal short arm of inv(Y) similar to those observed in normal males. To further characterize and compare the inv(Y) of the patient and her father, Southern analysis of *ZFY* and *TSPY* sequences was performed. Both probes detected hybridizing fragments of the same size in DNA of various restriction enzyme digestions from the patient, her father and normal control individuals, indicating that there were no apparent alterations of *ZFY* (not shown) and *TSPY* sequences (Fig. 3b).

The copy number of DSS on X chromosomes in the patient and her father was examined by Southern analysis of the DAX-1 gene. As shown in Fig. 4, double dosage was only detected in the DNA of a normal female, and the intensity of the hybridizing band in DNA of the proband was consistent with that in DNA of her father and a normal male.

DISCUSSION

We undertook cytogenetic and molecular studies of a familial paracentrically inverted Y chromosome identified in a patient with ambiguous genitalia. In some patients, intersex abnormality is associated with sex chromosomal mosaicism, particularly 45,X [Wolman et al., 1985]. Cytogenetic analysis performed on cells from 3 different tissues, including both gonads, failed to detect 45,X and/or 46,XX cell lines in our patient. Thus, it is very unlikely that the abnormal sexual development in our patient is related to the presence of Y-chromosome mosaicism.

Studies in the past few years have clearly established the *SRY* gene to be the primary testis determining gene in humans [Sinclair et al., 1990; Koopman et al., 1991]. Mutations in the DNA-binding HMG box of the *SRY* gene have been demonstrated in several cases of XY females [Müller et al., 1992; Mittwoch, 1992]. Molecular studies of both the promoter and coding re-

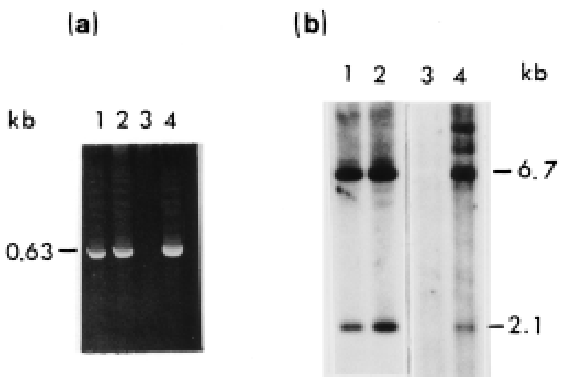

Fig. 3. PCR amplification of SRY gene (**a**) and Southern analysis of TSPY sequence (**b**). **Lane 1:** Proband; **lane 2:** father of proband; **lane 3:** normal female; **lane 4:** normal male.

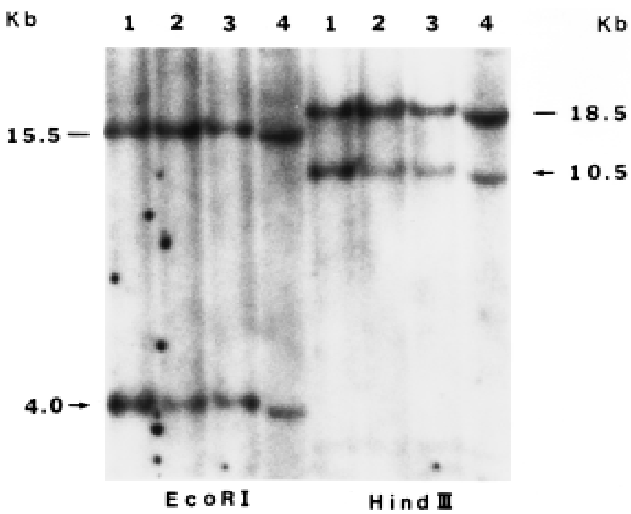

Fig. 4. Southern blotting dosage analysis of DAX-1 gene. The 15.5 Kb EcoRI and 18.5 Hind III fragments are of DAX-1 specific hybridization. A probe of phospholipase Cδ1 gene detected 4.0 Kb EcoRI and 10.5 Hind III bands serving as an internal control for the amount of DNA loaded in each lane. **Lane 1:** Proband; **lane 2:** father of proband; **lane 3:** normal male; **lane 4:** normal female. Disometry analysis indicated that the ratios of bands 4.0Kb:15.5Kb (DAX-1:phospholipase genes) are 1.49, 0.7, 0.81 and 0.74 and of bands 10.5Kb:18.5Kb(DAX-1:phospholipase genes) are 0.87, 0.45, 0.48 and 0.37 for lanes 1-4 respectively.

gions suggested that the *SRY* gene in the proband appears to be the same as that in her father and normal control individuals. Sequencing of the coding region further indicated that our patient harbors a normal *SRY* gene. Additional studies of *ZFY* and *TSPY* gene sequences revealed that the short arm of her inv(Y) chromosome is identical to that of her father.

Although mutations in the *SRY* gene have been identified in a few cases of XY females, there seem to be no detectable mutations within the *SRY* gene of this patient as well as many other XY females [Mittwoch, 1992; Bogan and Page, 1994]. One possible explanation is that mutations in other genes involved in sex differentiation, such as those postulated to be on either X or autosomes, may be associated with the abnormal sexual development. There may be more than one such X-linked genes but DAX-1 has so far been the only one identified. The male hybridization pattern of the DAX-1 gene suggested that the phenotype of our patient was not due to alterations at the DSS locus.

The molecular etiology of the intersex abnormality in our patient who seemingly has the same inv(Y) as her father and grandfather is extremely intriguing. The paracentric inversion in the long arm of the Y chromosome is extremely rare and its clinical significance is unknown. The familial occurrence of this inv(Y) in the present case suggests that it probably has no phenotypic effects. The concurrence of ambiguous genitalia and the inv(Y) described could be merely coincidental. *SRY* expression possibly abolished by long-range position effects of other Y sequences has been shown in XY female mice [Capel et al., 1993]. In our patient, alternatively, some types of positional effects of the paracentric inversion in the long arm might have affected the normal expression of the *SRY* gene during embryonic development, thus, the testis determining effect of the *SRY* gene had not been fully realized. Since her father and paternal grandfather developed normally into fertile males, such spurious *SRY* gene expression would be absent during their embryonic development. At present, it is difficult to evaluate this hypothesis. Studies of other sex reversed patients with normal *SRY* genes and/or similar inverted Y chromosomes will shed additional light on the possibility of such positional effects on *SRY* gene expression and testis determination.

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